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of

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for

**MODIFIED SIPHONS FOR IMPROVING
METERING PRECISION**

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1 The present application is a continuation of U.S. Patent Application Serial No.
2 08/562,327, which is a continuation of U.S. Patent Application Serial No. 08/254,406,
3 which is a continuation in part of U.S. Patent Application Serial No. 08/115,162, each of
4 which is incorporated herein by reference.

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6 **BACKGROUND OF THE INVENTION**

7 The present invention relates generally to devices and methods for analyzing
8 biological fluids. In particular, it relates to the design and use of improved centrifugal
9 rotors having siphons which allow delivery of a precise volume of liquid to a chamber in
10 the rotor.

11 Biological tests of blood plasma and other biological fluids frequently require that
12 fluids be quickly divided into predetermined volumes for analysis in a variety of optical
13 tests or assays. It is also frequently desirable to separate potentially interfering cellular
14 components of the material from the other fluid prior to testing. Such measurement and
15 separation steps have previously been typically performed by centrifugation to separate, for
16 instance, blood plasma from the cellular components, followed by manual or automated
17 pipetting of predetermined volumes of the blood plasma into separate test walls. Such
18 procedures are labor intensive and time-consuming. As a result, various automated
19 systems and methods have been proposed for providing multiple aliquots of plasma
20 suitable for testing in a more efficient manner.

21 A major advance in the analysis of biological fluids has been the use of centrifugal
22 rotors. These rotors are designed to measure volumes of a biological fluid, such as blood,
23 remove cellular components, and mix the fluid with an appropriate diluents for analysis,
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1 for example by optical testing. Typically, the rotors provide a plurality of discrete volumes
2 of sample in separate cuvettes in which the sample is optically analyzed.

3 To ensure accurate and consistent results, such rotors require the delivery of
4 precisely measured volumes of liquid to various chambers in the rotor. This must often be
5 accomplished in circumstances in which the rotor quickly accelerates and decelerates or is
6 otherwise perturbed during operation. This perturbation can often lead to delivery of
7 inaccurately measured volumes. The present invention addresses these and other needs.

8 9 Description of the Background Art

10 U.S. Patent Nos. 4,894,204 and 5,160,702 disclose siphons for transferring fluids
11 between chambers in a rotor. U.S. Patent No. 4,244,916 discloses a rotor comprising a
12 plurality of cuvettes positioned radially outward of a central receptacle. Each cuvette is
13 connected to the central receptacle by a duct and comprises a separate air escape orifice.
14 U.S. Patent No. 4,314,968 relates to rotors having cells positioned on the periphery of the
15 rotor. Each cell includes a peripheral orifice for removing fluid introduced into the cell.
16 U.S. Patent No. 4,902,479 discloses a multicuvette rotor comprising elongated, radially
17 extending cuvettes. Each elongated cuvette comprises a first chamber for receiving a first
18 constituent and a second chamber for receiving a second constituent. A divider structure
19 between the first and second chambers prevents mixing of the constituents before a
20 predetermined time. Mixing occurs as the rotor is spun at a significant speed. U.S. Patent
21 No. 4,963,498 discloses devices which rely upon capillaries, chambers, and orifices to
22 pump and mix fluids for optical analysis. U.S. Patent No. 5,077,013 discloses rotors
23 comprising peripheral cuvettes connected to holding chambers positioned radially inward
24 from the cuvettes.

SUMMARY OF THE INVENTION

The present invention provides centrifugal rotors comprising siphons for delivering a premeasured volume of liquid, typically a biological sample such as plasma, between a first and a second chamber in the rotor. The siphons of the invention have an elbow that is radially inward of the radially most inward point of the fluid in the first chamber. As the rotor is spinning the fluid does not flow past the elbow. After the rotor stops, capillary forces "prime" the siphon by pulling fluid just around the elbow. When the rotor is restarted, centrifugal force draws the remaining fluid out of the metering chamber into the receiving chamber until the level of the fluid in the metering chamber is at the same radial distance as the outlet of the siphon. The siphons of the invention are designed such that the inlet of the siphon on the first chamber is radially outward ^{or inward} of the siphon outlet on the second chamber.

The positioning of the inlets and outlets of the siphons of the invention provide a number of advantages. For example, the inlet of the siphon is always positioned radially outward of the final position of the meniscus of the fluid in the first chamber, after fluid has been transferred to the second chamber. Thus, inaccuracy in measurement associated with different shaped menisci in different fluids is minimized since the meniscus is minimized. ~~In addition, one of skill will recognize that all siphons are semi-stable because the train of fluid in a siphon is stable but easily broken if the rotor is perturbed. When the train of fluid is broken, under centrifugal force, the fluid contained in the siphon will flow to the radially most outward point. In prior art siphons this point is the siphon outlet. Thus, the potential exists for the delivery of unmetered volumes of fluid to the receiving chamber. In the siphons of the present invention, the radially most outward point in the siphon is the siphon inlet. In this design, the problem of delivering unmetered volumes of~~

1 fluid is avoided because the fluid flows back into the first chamber when the train of fluid
2 is broken.

3 The chambers connected by the siphons of the invention are used to perform any of
4 a number of functions, such as metering liquids, separating solid components from a
5 sample, mixing diluent with the sample, and the like. In the preferred embodiments, the
6 siphons connect a plasma metering chamber to a mixing chamber for mixing the
7 premeasured volume of plasma with diluent.

8 In addition, the rotors of the invention comprise unmodified inlet channels
9 connecting a distribution ring to cuvettes comprising reagents for optical analysis of a
10 biological sample. The inlet channels are sized such that, as the rotor spins, gas escapes
11 from the cuvette through the inlet channel as the liquid enters the cuvette through the inlet
12 channel. An "unmodified inlet channel" as used herein refers to a simple inlet channel,
13 typically having a rectangular cross section, which is not modified (e.g., by altering the
14 cross-sectional shape, surface texture, and the like) to provide a pathway for gas to escape
15 from a cuvette that is not otherwise vented.

BRIEF DESCRIPTION OF THE DRAWINGS

Figs. 1A-1F are top plan views of a rotor of the invention showing the flow of fluids through the chambers and channels of the rotor as the rotor is spun.

DESCRIPTION OF THE PREFERRED EMBODIMENT

The present invention provides methods and devices for the delivery of liquids to chambers in an analytical rotor. The rotors of the invention comprise siphons which ensure precise delivery of metered volumes of liquid to a desired chamber in the rotor.

The rotors of the invention are suitable for the analysis of any liquid, typically a biological sample such as whole blood or plasma. It is also useful with numerous other biological fluids, such as urine, sputum, semen, saliva, ocular lens fluid, cerebral fluid, spinal fluid, amniotic fluid. Other fluids that can be tested include tissue culture media, food and industrial chemicals, environmental samples and the like.

The rotors typically provide chambers which can separate cellular components from the biological sample (e.g. whole blood), measure a precise volume of liquid sample (e.g. plasma), mix the sample with an appropriate diluent and deliver the diluted sample to cuvettes for optical analysis. The fluid delivered to the cuvettes, undergoes reaction(s) within the cuvettes, e.g., reaction with a reagent which forms part of an analytical procedure to detect one or more analytes within the fluid. The sample may further be optically analyzed while present in the rotor, either with or without prior reaction.

The apparatus of the present invention comprises an analytical rotor having a rotor body which is capable of being mounted on a conventional laboratory centrifuge of the type which is commercially available from suppliers, such as Beckman Instruments, Inc., Spinco Division, Fullerton, California; Fisher scientific, Pittsburgh, Pennsylvania; VWR Scientific, San Francisco, California, and the like. Generally, the centrifugal rotor will include a receptacle or other coupling device suitable for mounting on a vertical drive shaft provided by the centrifuge. The particular design of the receptacle or coupling device will depend on the nature of the centrifuge, and it will be appreciated that the centrifugal rotor

1 of the present invention may be adapted for use with all or most types of centrifuges which
2 are now available or which may become available in the future.

3 The rotor body comprises a structure which maintains a desired geometric pattern
4 or relationship between a plurality of chambers, interconnection passages, and vents, as
5 described in more detail below. Various specialized chambers and channels suitable for
6 use in the rotors of the invention are disclosed in U.S. Patent Nos. 5,061,381 and
7 5,122,284, and U.S.S.N. 07/678,762 and 07/783,041 which are incorporated herein by
8 reference.

9 Usually, the body will be a substantially solid plate or disk with the chambers and
10 passages formed as spaces or voids in the otherwise solid matrix. Conveniently, such solid
11 plate structures may be formed by laminating a plurality of separately-formed layers
12 together into a composite structure where the chambers and horizontal passages are
13 generally formed between adjacent layers. The vertical passages may be formed through
14 the layers. The individual layers may be formed by injection molding, machining, or
15 combinations thereof, and will usually be joined together, typically using a suitable
16 adhesive or by ultrasonic welding. The final enclosed volumes are formed when the layers
17 are brought together.

18 Of course, the centrifugal rotor could also be formed as a plurality of discrete
19 components, such as tubes, vessels, chambers, etc., arranged in a suitable framework.
20 Such assemblies of discrete components, however, are generally more difficult to
21 manufacture and are therefore less desirable than those formed within a substantially solid
22 plate.

23 The rotor body may be formed from a wide variety of materials and may optionally
24 include two or more materials. Usually, the material(s) will be transparent so that the

1 presence and distribution of the biological fluid, cellular components, and reagents may be
2 observed within the various internal chambers and passages. Optionally, to the extent
3 analytical chambers, e.g., cuvettes, or other test wells are formed within the rotor, it is
4 desirable to have suitable optical paths formed within the rotor so that the contents of the
5 cuvettes may be observed spectrophotometrically, fluorometrically, or by other optical
6 assessment instruments. The construction of suitable cuvettes having particular optical
7 paths formed therethrough is disclosed in U.S. Patent No. 5,173,193, the disclosure of
8 which is incorporated herein by reference. In the preferred embodiment, the rotor is
9 formed with an acrylic resin having suitable optical properties, at least in those areas which
10 define an optical path.

11 The apparatus and method of the present invention are suitable for performing a
12 wide variety of analytic procedures and assays which are beneficially or necessarily
13 performed on blood plasma and other samples. The analytic procedures may require that
14 the sample be combined with one or more reagents so that some detectable change occurs
15 which may be related to the presence and/or amount of a particular component (analyte) or
16 characteristic of the sample. For instance, the sample may undergo a reaction or other
17 change which results in a change in color, fluorescence, luminescence, or the like, which
18 may be measured by conventional spectrophotometers, fluorometers, light detectors, and
19 the like. In some cases, immunoassays and other specific binding assays may be
20 performed within the cell-free fluid collection chamber or within cuvettes which are
21 connected to the collection chamber. Generally, such assay procedures should be
22 homogeneous and not require a separation step. In other cases, however, it may be
23 possible to accommodate heterogeneous assay systems by providing a means to separate
24 the sample (e.g., blood plasma) from the collection chamber or another test well or cuvette

1 after the immunological reaction step has occurred. One of skill will recognize that the
2 means of analyzing the sample is not an important aspect of the invention. Any of a
3 number of analytical methods can be adapted for use in the rotors of the invention,
4 depending upon the particular sample being analyzed and component being detected.

5 In the case of blood analyses, conventional blood assays are typically performed.
6 Examples of assays which may be performed include those designed to detect glucose,
7 lactate, dehydrogenase, serum glutamic-oxaloacetic transaminase (SGOT), serum
8 glutamic-pyruvic transaminase (SGPT), blood urea nitrogen (BUN), total protein,
9 alkalinity, phosphatase, bilirubin, calcium, chloride, sodium, potassium, magnesium, and
10 the like. This list is not exhaustive and is intended merely as being exemplary of the
11 assays which may be performed using the apparatus and method of the present invention.
12 Usually, these tests will require that the blood and plasma be combined with one or more
13 reagents which result in an optically detectable, usually photometrically detectable, change
14 in the plasma. The reagents which are required are well known and amply described in the
15 patent and scientific literature.

16 The reagents are preferably provided in lyophilized form to increase stability.
17 Ideally, they are provided in the form of lyophilized reagent spheres as described in
18 U.S.S.N. 07/747,179, which is incorporated herein by reference.

19 Referring now to Figures 1A-F, an analytical rotor comprising the chambers and
20 channels of the present invention can be seen. Figure 1A shows the position of a blood
21 sample 102 in the blood application chamber 104 after the sample has been loaded in the
22 rotor body 100. A diluent container in chamber 106 is opened upon mounting of the rotor
23 on the spindle of the centrifuge as described in copending and commonly assigned
24 application, U.S. Serial No. 07/873,327, which is incorporated herein by reference.

1 Fig. 1B shows the position of the diluent 108 and blood sample 102 after the rotor
2 is spun at 4,000 rpm. The blood sample 102 begins to exit the blood application chamber
3 104 and enters the plasma metering chamber 110. At the same time, diluent 108 empties
4 from the diluent container into the holding chamber 112. The diluent immediately begins
5 to enter the diluent metering chamber 114 through channel 116.

6 Fig. 1C shows the position of the liquids as the rotor 100 continues to spin. Here,
7 the blood sample 102 has emptied the blood application chamber 104 and overflows the
8 plasma metering chamber 110 into the overflow chamber 118 where it flows to the
9 hemoglobin cuvette 120 and the excess blood dump 122. Meanwhile, diluent 108 fills the
10 diluent metering chamber 114 and excess flows through channel 124 to diluent-only
11 cuvettes 126 and excess diluent dump 127.

12 Fig. 1D shows the position of the liquids at the conclusion of the first spin. The
13 blood sample 102 has separated into cells 128 and plasma 130. The diluent-only cuvettes
14 126 are filled and a predetermined amount of diluent remains in the diluent metering
15 chamber 114. The rotor 100 is then stopped and the siphon 132 from the diluent metering
16 chamber 114, as well as the siphon 134 from the plasma metering chamber 110, are
17 allowed to prime, as described above. Siphon 134 is a siphon of the present invention. It
18 is connected to the plasma metering chamber 110 at inlet 138. The inlet 138 is positioned
19 radially outward of the siphon outlet 139, through which the siphon 134 empties into the
20 mixing chamber 136.

21 Fig. 1E shows the position of the liquids during the second spin of the rotor. The
22 diluent metering chamber 114 empties into the mixing chamber 136 through siphon 132.
23 A predetermined amount of plasma 130 is metered into the mixing chamber 136 and the
24 two fluids are mixed, thereby forming diluted plasma 131. The amount of plasma 130

1 delivered to the mixing chamber 136 is determined by the position of the outlet 139 on the
2 siphon 134. As can be seen in this figure, the final level of the plasma 133 in the plasma
3 metering chamber 110 is at the same radial position as the outlet 139. Thus, the volume of
4 plasma delivered to the mixing chamber 136 is determined by the volume of the plasma
5 metering chamber 110 between the exit to the overflow chamber 129 and the final level of
6 plasma 133. After the plasma and diluent are mixed in the mixing chamber 136, the rotor
7 is stopped again and the output siphon 140 is primed.

8 Fig. 1F shows the position of the diluted plasma 131 as the rotor is spun during the
9 third spin. This figure illustrates the movement of the diluted plasma 131 through the
10 distribution ring 142 and inlet channels 144 to the cuvettes 146 and excess plasma dump
11 147. The resistance to flow in the output siphon 140 is selected to be higher than the
12 resistance to flow in the distribution ring 142 and the inlet channels 144 so that air present
13 in the cuvettes 146 can escape as the cuvettes are filled. Specifically, siphon 140 is
14 dimensioned such that the ratio of the cross sectional area of the inlet channels 144 to the
15 cross sectional area of the liquid in them is greater than 2:1, preferably greater than about
16 4:1. The cross sectional area of the inlet channels 144 is typically the same as or slightly
17 smaller than that of the distribution channel 142 so that gas in the unvented cuvettes
18 escapes through the inlet channels 144 and distribution 142. If the sample is plasma or
19 diluted plasma and the channels are rectangular in cross-section, their dimensions are
20 typically as follows: siphon: 0.150 mm depth, 0.200 mm width; distribution channel 0.300
21 mm depth, 0.5 mm width; inlet channels: 0.150 depth, 0.500 width.

22 After the cuvettes have been filled, reagents present in the cuvettes are mixed with
23 the solution and the necessary photometric analyses are made on the sample. Such
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analyses are carried out as described above according to methods known to those of skill in the art.

Although the foregoing invention has been described in detail for purposes of clarity of understanding, it will be obvious that certain modifications may be practiced within the scope of the appended claims.